10/024,997

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Search Results - Record(s) 1 through 3 of 3 returned.

☐ 1. Document ID: US 20020150536 A1

L2: Entry 1 of 3 File: PGPB Oct 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020150536
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020150536 A1

TITLE: Nucleic acid ligands to integrins

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ruckman, Judy	Boulder	CO	US	
Gold, Larry	Boulder	CO	US	
Stephens, Andrew	Boulder	CO	US	
Janjic, Nebojsa	Boulder	CO	US	

US-CL-CURRENT: 424/1.73; 435/6, 514/44

ABSTRACT:

Methods are described for the isolation of nucleic acid ligands to integrins using the SELEX process. SELEX is an acronym for Systematic Evolution of Ligands by EXponential enrichment. The nucleic acid ligands of the present invention are useful as therapeutic and diagnostic agents.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☐ 2. Document ID: US 6331394 B1

L2: Entry 2 of 3 File: USPT Dec 18, 2001

US-PAT-NO: 6331394
DOCUMENT-IDENTIFIER: US 6331394 B1

TITLE: Nucleic acid ligands to integrins

DATE-ISSUED: December 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ruckman; Judy	Boulder	CO		
Gold; Larry	Boulder	CO		
Stephens; Andrew	Boulder	CO		
Janjic; Nebojsa	Boulder	CO		

US-CL-CURRENT: 435/6; 435/91.2, 536/23.1, 536/25.4

ABSTRACT:

Methods are described for the isolation of nucleic acid ligands to integrins using the SELEX process. SELEX is an acronym for Systematic Evolution of Ligands by EXponential enrichment. The nucleic acid ligands of the present invention are useful as therapeutic and diagnostic agents.

19 Claims, 8 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6

☐ 3. Document ID: WO 200109159 A1 AU 200062349 A US 6344321 B1 EP 1203007 A1

L2: Entry 3 of 3 File: DWPI Feb 8, 2001

DERWENT-ACC-NO: 2001-103180
DERWENT-WEEK: 200281

TITLE: Isolation of nucleic acid ligands to hepatocyte growth factor, its receptor c-met and integrins, useful for treating tumors, deep vein thrombosis and diabetic retinopathy

INVENTOR: GOLD, L; JANJIC, N ; LOCHRIE, M ; RABIN, R ; RUCKMAN, J ; STEPHENS, A

PRIORITY-DATA: 1999US-0364543 (July 29, 1999), 1999US-0364539 (July 29, 1999), 1990US-0536428 (June 10, 1990), 1991US-0714131 (June 10, 1991), 1995US-0469609 (June 6, 1995), 1998US-0502344 (August 27, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 200109159 A1	February 8, 2001	E	078	C07H021/04
AU 200062349 A	February 19, 2001		000	C07H021/04
US 6344321 B1	February 5, 2002		000	C07H021/04
EP 1203007 A1	May 8, 2002	E	000	C07H021/04

INT-CL (IPC): A61 K 48/00; C07 H 21/02; C07 H 21/04; C12 N 15/85; C12 N 15/86; C12 P 19/34; C12 Q 1/68

ABSTRACTED-PUB-NO: US 6344321B
BASIC-ABSTRACT:

NOVELTY - A method (M1) for the isolation of nucleic acid ligands to X comprising:

(a) contacting a candidate mixture of nucleic acids with X;

(b) partitioning nucleic acids with an increased affinity to X from the remainder of the mixture; and

(c) amplifying the increased affinity nucleic acids to yield nucleic acids with higher affinity and binding specificity for X, is new.

DETAILED DESCRIPTION - A method (M1) for the isolation of nucleic acid ligands to hepatocyte growth factor/scatter factor (HGF), c-met or an integrin comprising:

(a) contacting a candidate mixture of nucleic acids with HGF, c-met or an integrin;

(b) partitioning nucleic acids with an increased affinity to HGF, c-met or an integrin relative to the candidate mixture, from the remainder of the mixture; and

(c) amplifying the increased affinity nucleic acids to yield a nucleic acid mixture enriched for nucleic acids with higher affinity and binding specificity to HGF, c-met or an integrin, where a nucleic acid ligand to HGF, c-met or an integrin may be identified, is new.

INDEPENDENT CLAIMS are also included for the following:

(1) a (purified and isolated non-naturally occurring) nucleic acid ligand (I) to HGF identified by (M1);

(2) a purified and non-naturally occurring RNA ligand to HGF comprising a fully defined nucleotide sequence given in the specification;

(3) determining the level of HGF in an individual comprising contacting a biological fluid from the individual with (I) and determining the amount of HGF bound to (I);

(4) a (purified and isolated non-naturally occurring) nucleic acid ligand (II) to c-met identified by (M1);

(5) a purified and non-naturally occurring RNA ligand to c-met comprising a fully defined nucleotide sequence given in the specification;

(6) a (purified and isolated non-naturally occurring) nucleic acid ligand (III) to an integrin identified by (M1); and

(7) a purified and non-naturally occurring RNA ligand to an integrin comprising a fully defined nucleotide sequence given in the specification.

ACTIVITY - Cytostatic; thrombolytic; anticoagulant; antidiabetic; antirheumatic; antiarthritic; cerebroprotective; osteopathic; vasotropic; gynecological; antipsoriatic.

MECHANISM OF ACTION - Inhibitors of HGF (hepatocyte growth factor/scatter factor), c-met, integrins, VEGF (vascular endothelial growth factor) and/or bFGF (basic fibroblast growth factor). No supporting data given.

USE - Nucleic acid ligands (I) to hepatocyte growth factor/scatter factor (HGF) are useful for treating diseases in which elevated HGF is a causative factor. (I) and nucleic acid ligands (II) to c-met are useful for treating tumors and for inhibiting angiogenesis. Optionally (I) may be used in combination with a nucleic acid ligand to vascular endothelial growth factor (VEGF) and/or basic fibroblast growth factor (bFGF) to inhibit tumor development. Nucleic acid ligands to at least 2 growth factors (e.g. VEGF, platelet derived growth factor, transforming growth factor beta , HGF and keratinocyte growth factor) are also useful for inhibiting tumor development. A nucleic acid ligand (III) to beta 3 integrin is useful for treating a disease resulting from platelet activation and deep vein thrombosis. It is also useful for detecting a deep vein thrombosis in an individual comprising administrating (III) conjugated to a radioactive label to the individual and detecting the site of the thrombosis by analyzing the localization of (III) using a radio imaging technique. (III) is useful in an anti-clotting composition for use in acute coronary syndromes and percutaneous coronary intervention. Nucleic acid ligands to alpha v beta 3 are useful for treating a disease in which alpha v beta 3 activation is a contributing pathogenic factor such as cancer, diabetic retinopathy, retinopathy of maturity, macular degeneration, endometriosis, psoriasis, rheumatoid arthritis, stroke, osteoporosis and restenosis (all claimed).
ABSTRACTED-PUB-NO:

WO 200109159A EQUIVALENT-ABSTRACTS:

NOVELTY - A method (M1) for the isolation of nucleic acid ligands to X comprising:

(a) contacting a candidate mixture of nucleic acids with X;

(b) partitioning nucleic acids with an increased affinity to X from the remainder of the mixture; and

(c) amplifying the increased affinity nucleic acids to yield nucleic acids with higher affinity and binding specificity for X, is new.

DETAILED DESCRIPTION - A method (M1) for the isolation of nucleic acid ligands to hepatocyte growth factor/scatter factor (HGF), c-met or an integrin comprising:

(a) contacting a candidate mixture of nucleic acids with HGF, c-met or an integrin;

(b) partitioning nucleic acids with an increased affinity to HGF, c-met or an integrin relative to the candidate mixture, from the remainder of the mixture; and

(c) amplifying the increased affinity nucleic acids to yield a nucleic acid mixture enriched for nucleic acids with higher affinity and binding specificity to HGF, c-met or an integrin, where a nucleic acid ligand to HGF, c-met or an integrin may be identified, is new.

INDEPENDENT CLAIMS are also included for the following:

(1) a (purified and isolated non-naturally occurring) nucleic acid ligand (I) to HGF identified by (M1);

(2) a purified and non-naturally occurring RNA ligand to HGF comprising a fully defined nucleotide sequence given in the specification;

(3) determining the level of HGF in an individual comprising contacting a biological fluid from the individual with (I) and determining the amount of HGF bound to (I);

(4) a (purified and isolated non-naturally occurring) nucleic acid ligand (II) to c-met identified by (M1);

(5) a purified and non-naturally occurring RNA ligand to c-met comprising a fully defined nucleotide sequence given in the specification;

(6) a (purified and isolated non-naturally occurring) nucleic acid ligand (III) to an integrin identified by (M1); and

(7) a purified and non-naturally occurring RNA ligand to an integrin comprising a fully defined nucleotide sequence given in the specification.

ACTIVITY - Cytostatic; thrombolytic; anticoagulant; antidiabetic; antirheumatic; antiarthritic; cerebroprotective; osteopathic; vasotropic; gynecological; antipsoriatic.

MECHANISM OF ACTION - Inhibitors of HGF (hepatocyte growth factor/scatter factor), c-met, integrins, VEGF (vascular endothelial growth factor) and/or bFGF (basic fibroblast growth factor). No supporting data given.

USE - Nucleic acid ligands (I) to hepatocyte growth factor/scatter factor (HGF) are useful for treating diseases in which elevated HGF is a causative factor. (I) and nucleic acid ligands (II) to c-met are useful for treating tumors and for inhibiting angiogenesis. Optionally (I) may be used in combination with a nucleic acid ligand to vascular endothelial growth factor (VEGF) and/or basic fibroblast growth factor (bFGF) to inhibit tumor development. Nucleic acid ligands to at least 2 growth factors (e.g. VEGF, platelet derived growth factor, transforming growth factor beta , HGF and keratinocyte growth factor) are also useful for inhibiting tumor development. A nucleic acid ligand (III) to beta 3 integrin is useful for treating a disease resulting from platelet activation and deep vein thrombosis. It is also useful for detecting a deep vein thrombosis in an individual comprising administrating (III) conjugated to a radioactive label to the individual and detecting the site of the thrombosis by analyzing the localization of (III) using a radio imaging technique. (III) is useful in an anti-clotting composition for use in acute coronary syndromes and percutaneous coronary intervention. Nucleic acid ligands to alpha v beta 3 are useful for treating a disease in which alpha v beta 3 activation is a contributing pathogenic factor such as cancer, diabetic retinopathy, retinopathy of maturity, macular degeneration, endometriosis, psoriasis, rheumatoid arthritis, stroke, osteoporosis and restenosis (all claimed).

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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Terms	Documents
L1 with (platelet or thrombosis)	3

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Search Results - Record(s) 1 through 6 of 6 returned.

☐ 1. Document ID: US 20020150536 A1

L3: Entry 1 of 6 File: PGPB Oct 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020150536
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020150536 A1

TITLE: Nucleic acid ligands to integrins

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ruckman, Judy	Boulder	CO	US	
Gold, Larry	Boulder	CO	US	
Stephens, Andrew	Boulder	CO	US	
Janjic, Nebojsa	Boulder	CO	US	

US-CL-CURRENT: 424/1.73; 435/6, 514/44

ABSTRACT:

Methods are described for the isolation of nucleic acid ligands to integrins using the SELEX process. SELEX is an acronym for Systematic Evolution of Ligands by EXponential enrichment. The nucleic acid ligands of the present invention are useful as therapeutic and diagnostic agents.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

☐ 2. Document ID: US 20020115629 A1

L3: Entry 2 of 6 File: PGPB Aug 22, 2002

PGPUB-DOCUMENT-NUMBER: 20020115629
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020115629 A1

TITLE: Aptamer-mediated regulation of gene expression

PUBLICATION-DATE: August 22, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ramachandra, Murali	San Diego	CA	US	

US-CL-CURRENT: 514/44; 536/23.1

ABSTRACT:

This invention provides methods of regulating gene expression. An aptamer is positioned in a nucleic acid molecule along with a sequence encoding a transcriptional regulatory polypeptide. The aptamer disrupts translation of the transcriptional regulatory polypeptide when contacted with an aptamer-binding ligand. Gene expression levels can be either increased or decreased by the disclosed methods, depending on whether the transcriptional regulatory polypeptide is a repressor or activator, and the degree of the effect is dependent upon the dose of the ligand. Nucleic acid molecules, expression cassettes, expression vectors and cells useful in the gene regulation methods are also provided.

☐ 3. Document ID: US 20020006401 A1

L3: Entry 3 of 6 File: PGPB Jan 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020006401
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020006401 A1

TITLE: Modulation of vascular healing by inhibition of leukocyte adhesion and function

PUBLICATION-DATE: January 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Rogers, Campbell	Westwood	MA	US	
Edelman, Elazer R.	Brookline	MA	US	
Simon, Daniel I.	Waban	MA	US	

US-CL-CURRENT: 424/130.1

ABSTRACT:

Compounds that specifically inhibit or reduce leukocyte adhesion or function are useful to enhance vascular healing and lessen restenosis of blood vessels after revascularization, via angioplasty or bypass surgery, of diseased coronary, peripheral and cerebral arteries, and lessen stenosis or restenosis of surgically-placed bypass grafts and transplanted organs. Examples of these compounds are those which block cell surface integrins or their ligands, for example, the leukocyte integrin Mac-1 (CD11b/CD18, .alpha.M.beta.2). As demonstrated by the examples, both superficial and deep injury was significantly reduced with treatment using an antibody to Mac-1 compared to both saline controls and IgG controls. After balloon angioplasty (superficial injury) neointimal area was reduced nearly 70%. The ratio of intimal:medial area, which is customarily used in balloon-injured experimental arteries to normalize for small normal variations in arterial size from one animal to another, was reduced over 75%. After endovascular stent implantation (deep injury) neointimal area was reduced nearly 40%. Extrapolated to humans, this reduction in the intimal thickening would reduce restenosis from occurring in approximately 30% of patients to less than 10% of patients.

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4. Document ID: US 6331394 B1

L3: Entry 4 of 6 File: USPT Dec 18, 2001

US-PAT-NO: 6331394
DOCUMENT-IDENTIFIER: US 6331394 B1

TITLE: Nucleic acid ligands to integrins

DATE-ISSUED: December 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ruckman; Judy	Boulder	CO		
Gold; Larry	Boulder	CO		
Stephens; Andrew	Boulder	CO		
Janjic; Nebojsa	Boulder	CO		

US-CL-CURRENT: 435/6; 435/91.2, 536/23.1, 536/25.4

ABSTRACT:

Methods are described for the isolation of nucleic acid ligands to integrins using the SELEX process. SELEX is an acronym for Systematic Evolution of Ligands by EXponential enrichment. The nucleic acid ligands of the present invention are useful as therapeutic and diagnostic agents.

19 Claims, 8 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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5. Document ID: US 6331394 B1

L3: Entry 5 of 6 File: DWPI Dec 18, 2001

DERWENT-ACC-NO: 2002-121160
DERWENT-WEEK: 200281

TITLE: Novel non-naturally occurring nucleic acid (RNA) ligand to a beta-3 type integrin, useful in the treatment of cancer and thrombosis

INVENTOR: GOLD, L; JANJIC, N ; RUCKMAN, J ; STEPHENS, A

PRIORITY-DATA: 2000US-0606477 (June 29, 2000), 1991US-0714131 (June 10, 1991), 1994US-0234997 (April 28, 1994), 1997US-0956699 (October 23, 1997), 1999US-0364543 (July 29, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 6331394 B1	December 18, 2001		050	C12Q001/68

INT-CL (IPC): C07 H 21/02; C07 H 21/04; C12 P 19/34; C12 Q 1/68

ABSTRACTED-PUB-NO: US 6331394B
BASIC-ABSTRACT:

NOVELTY - A new purified and isolated non-naturally occurring nucleic acid ligand (I) to an integrin.

DETAILED DESCRIPTION - (I) is preferably identified by a method (M1) comprising:

(1) preparing a candidate mixture of nucleic acids;

(2) contacting the candidate mixture of nucleic acids with the integrin, where the nucleic acids having an increased affinity to the integrin, relative to the candidate mixture, may be separated from the remainder of the candidate mixture;

(3) separating the increased affinity nucleic acids from the remainder of the candidate mixture; and

(4) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acids with relatively higher affinity and specificity for binding to the integrin, so that a nucleic acid ligand of the integrin may be identified.

An INDEPENDENT CLAIM is also included for the isolation of (I) comprising (M1) with isolation of (I) after its identification.

ACTIVITY - Cytostatic; thrombolytic; cardiant; antidiabetic; ophthalmological; antipsoriatic; gynecological.

No supporting data given.

MECHANISM OF ACTION - Nucleic acid ligand for integrins; alpha IIb beta 3 and alpha v beta 3 binding inhibitor.

To test whether aptamer 17.16 (a fully defined 84 RNA sequence given in the specification) could block the ligand binding site of alpha IIb beta 3 and alpha v beta 3, purified vitronectin and fibrinogen were biotinylated and incubated with one or both of the immobilized integrins in the presence or absence of varying concentrations of the aptamer or a non-binding control RNA.

This was done as follows: purified integrin ligands, vitronectin and fibrinogen, were biotinylated according to (Smith et al. (1990) J. Biol. Chem. 265:12267-71).

Biotinylated proteins were dialyzed into phosphate-buffered saline and 96-well microtiter plates were coated with either alpha IIb beta 3 or alpha v beta 3 and blocked with BSA. A fixed concentration of biotinylated ligand (fibrinogen: 6 nM final; vitronectin: 10 nM final) was pre-mixed in binding buffer with varying concentrations of aptamer, control RNA, cyclic RGD peptide, antibody, or unmodified ligand. The mixtures were incubated in the integrin-coated wells for 60 minutes at room temperature. After washing, bound biotinylated ligand was detected by addition of 100 micro l/well 1:500 dilution streptavidin-alkaline phosphatase conjugate (Calbiochem) (30 minutes at room temperature) followed by 100 micro l/well p-nitrophenyl phosphate.

Absorbance was read at 405 um. The data were fit to an equation that describes mutually exclusive binding of two ligands to a single target species (Gill et al. (1991) J. Mol. Biol. 220:307-24). The concentration of competitor that inhibited 50% of the maximum signal above background (IC50) was determined from the fitted curve.

Known ligand binding inhibitors, including an RGD peptide and the alpha v beta 3-specific antibody LM609, were included as positive controls for the assay.

Aptamer 17.16 inhibited the binding interaction with an IC50 of 4.7 nM while the control RNA showed no inhibition. By comparison, the IC50 of RGD peptide inhibition was 1.4 nM and that of LM609 was 2.7 nM. Unmodified vitronectin inhibited the binding of the biotinylated material with an IC50 of 59 nM. Similar data were obtained for aptamer inhibition of fibrinogen binding to alpha v beta 3 and for fibrinogen binding to alpha IIb beta 3. IC50 values for alpha v beta 3 inhibition were 17.16, 9.5 nM; control RNA, not measurable; RGD peptide, 1.0 nM; LM609, 6.3 nM; unmodified fibrinogen, 43 nM. IC50 values for alpha IIb beta 3 inhibition were: 17.16, 6.5 nM; control RNA, not measurable; RGD peptide, 21 nM; unmodified fibrinogen, 15 nM.

Aptamer 17.16 is an effective competitor of beta 3 integrin ligand binding and, on a molar basis, has an inhibitory potency nearly equivalent to that of a bivalent

antibody.

USE - (I) is especially useful as an inhibitor of alpha IIb beta 3 and alpha v beta 3 integrins and can be used to inhibit tumor growth and metastasis. They can also be used to treat ocular diseases including diabetic retinopathy, retinopathy of prematurity, and macular degeneration. Other diseases treated include endometriosis and psoriasis

In addition, (I) may useful in the treatment of thrombosis and cancer, and can be used as a diagnostic agent for thrombosis. (I) can also be used to treat acute coronary syndromes such as unstable angina or myocardial infarction.

Radiolabeled (I) to platelet-expressed integrins can be administered to individuals who are to undergo major surgery, or have suffered major trauma. (I) can function as imaging agents for the detection of thrombi, by showing sites in the body where large aggregations of platelets are present. If a thrombosis is detected by radioimaging at a critical site in the body, then anticoagulant and thrombolytic treatment, including treatment with (I), can be given locally. The advantage of using (I) as an imaging agent is that the anticoagulant and thrombolytic treatments, which can cause harm if administered prophylactically by allowing internal bleeding to continue without efficient clotting, can be given only to those individuals who definitely have a dangerous thrombosis. Moreover, these treatments can be specifically injected at the site where the thrombosis has been detected by the nucleic acid ligand, instead of injecting higher concentrations into the bloodstream in the hope that some active agent will be carried to all potential sites of thrombosis.

ADVANTAGE - (I), because of its specificity for the active, ligand-binding conformation of the integrin alpha IIb beta 3, may reduce the risk of bleeding complications associated with the existing anti-clotting therapies. Given the role of integrins in various disease states, the availability of high specificity inhibitors of integrins such as (I) is a particular advantage.

6. Document ID: US 20020151512 A1 DE 19502912 A1 EP 726274 A2 AU 9540747 A CA 2168409 A JP 08238093 A AU 711792 B US 6013639 A US 6121434 A EP 726274 B1 DE 59607853 G ES 2165443 T3

L3: Entry 6 of 6 File: DWPI Oct 17, 2002

DERWENT-ACC-NO: 1996-355223
DERWENT-WEEK: 200270

TITLE: Oligo:nucleotide(s) with series of G residues at at least one end have increased stability against nuclease and cell penetration, - are partic. anti:sense sequences for treating and diagnosing cancer, viral diseases etc.

INVENTOR: PEYMAN, A; UHLMANN, E

PRIORITY-DATA: 1995DE-1002912 (January 31, 1995)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 20020151512 A1	October 17, 2002		000	A61K048/00
DE 19502912 A1	August 1, 1996		015	C07H021/00
EP 726274 A2	August 14, 1996	G	026	C07H021/00
AU 9540747 A	August 8, 1996		000	C07H021/00
CA 2168409 A	August 1, 1996		000	C07H021/00
JP 08238093 A	September 17, 1996		014	C12N015/09
AU 711792 B	October 21, 1999		000	C12N015/11
US 6013639 A	January 11, 2000		000	A61K048/00
US 6121434 A	September 19, 2000		000	C07H021/04
EP 726274 B1	October 10, 2001	G	000	C07H021/00
DE 59607853 G	November 15, 2001		000	C07H021/00
ES 2165443 T3	March 16, 2002		000	C07H021/00

INT-CL (IPC): A61 K 31/70; A61 K 48/00; C07 H 21/00; C07 H 21/04; C12 N 15/09; C12 N 15/11; C12 Q 1/68

ABSTRACTED-PUB-NO: DE 19502912A
BASIC-ABSTRACT:

Oligonucleotides of formula (I) are new: 5'-CAP-(Oligo)-CAP-3' (I); Oligo = sequence of 10-40 nucleotides; CAP = Gm; m = 0-10, pref. 3-5, esp. 4; each m is same or different but not both zero.

USE - (I) are antisense, triplex-forming, aptamer or ribozyme molecules used to treat or diagnose viral disease, cancer, restenosis and diseases that involve (a) integrin or cell-cell adhesion receptors or (b) diffusible factors such as TNF alpha. (I) are directed against e.g. HIV, herpes simplex virus (HSV), influenza, hepatitis or papilloma viruses; nuclear or cytoplasmic-membrane associated oncogenes; cell receptors; cytokines; adhesion molecules etc..

ADVANTAGE - Addn. of CAP improves both stability against nuclease and cell penetration, so (I) have increased activity. They probably aggregate or associate but the resulting structure is in equilibrium with enough free (I) to control transcription or translation.
ABSTRACTED-PUB-NO:

EP 726274B EQUIVALENT-ABSTRACTS:

Oligonucleotides of formula (I) are new: 5'-CAP-(Oligo)-CAP-3' (I); Oligo = sequence of 10-40 nucleotides; CAP = Gm; m = 0-10, pref. 3-5, esp. 4; each m is same or different but not both zero.

USE - (I) are antisense, triplex-forming, aptamer or ribozyme molecules used to treat or diagnose viral disease, cancer, restenosis and diseases that involve (a) integrin or cell-cell adhesion receptors or (b) diffusible factors such as TNF alpha . (I) are directed against e.g. HIV, herpes simplex virus (HSV), influenza, hepatitis or papilloma viruses; nuclear or cytoplasmic-membrane associated oncogenes; cell receptors; cytokines; adhesion molecules etc..

ADVANTAGE - Addn. of CAP improves both stability against nuclease and cell penetration, so (I) have increased activity. They probably aggregate or associate but the resulting structure is in equilibrium with enough free (I) to control transcription or translation.

US 6013639A

Oligonucleotides of formula (I) are new: 5'-CAP-(Oligo)-CAP-3' (I); Oligo = sequence of 10-40 nucleotides; CAP = Gm; m = 0-10, pref. 3-5, esp. 4; each m is same or different but not both zero.

USE - (I) are antisense, triplex-forming, aptamer or ribozyme molecules used to treat or diagnose viral disease, cancer, restenosis and diseases that involve (a) integrin or cell-cell adhesion receptors or (b) diffusible factors such as TNF alpha . (I) are directed against e.g. HIV, herpes simplex virus (HSV), influenza, hepatitis or

papilloma viruses; nuclear or cytoplasmic-membrane associated oncogenes; cell receptors; cytokines; adhesion molecules etc..

ADVANTAGE - Addn. of CAP improves both stability against nuclease and cell penetration, so (I) have increased activity. They probably aggregate or associate but the resulting structure is in equilibrium with enough free (I) to control transcription or translation.

US 6121434A

Oligonucleotides of formula (I) are new: 5'-CAP-(Oligo)-CAP-3' (I); Oligo = sequence of 10-40 nucleotides; CAP = Gm; m = 0-10, pref. 3-5, esp. 4; each m is same or different but not both zero.

USE - (I) are antisense, triplex-forming, aptamer or ribozyme molecules used to treat or diagnose viral disease, cancer, restenosis and diseases that involve (a) integrin or cell-cell adhesion receptors or (b) diffusible factors such as TNF alpha . (I) are directed against e.g. HIV, herpes simplex virus (HSV), influenza, hepatitis or papilloma viruses; nuclear or cytoplasmic-membrane associated oncogenes; cell receptors; cytokines; adhesion molecules etc..

ADVANTAGE - Addn. of CAP improves both stability against nuclease and cell penetration, so (I) have increased activity. They probably aggregate or associate but the resulting structure is in equilibrium with enough free (I) to control transcription or translation.

US20020151512A

Oligonucleotides of formula (I) are new: 5'-CAP-(Oligo)-CAP-3' (I); Oligo = sequence of 10-40 nucleotides; CAP = Gm; m = 0-10, pref. 3-5, esp. 4; each m is same or different but not both zero.

USE - (I) are antisense, triplex-forming, aptamer or ribozyme molecules used to treat or diagnose viral disease, cancer, restenosis and diseases that involve (a) integrin or cell-cell adhesion receptors or (b) diffusible factors such as TNF alpha . (I) are directed against e.g. HIV, herpes simplex virus (HSV), influenza, hepatitis or papilloma viruses; nuclear or cytoplasmic-membrane associated oncogenes; cell receptors; cytokines; adhesion molecules etc..

ADVANTAGE - Addn. of CAP improves both stability against nuclease and cell penetration, so (I) have increased activity. They probably aggregate or associate but the resulting structure is in equilibrium with enough free (I) to control transcription or translation.

WEST Search History

DATE: Sunday, December 29, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=OR			
L5	L4 with (aptamer or nucleic adj2 ligand)	3	L5
L4	integrin with (thrombosis or coronary or clot$4 or platelet)	1032	L4
L3	integrin with aptamer	6	L3
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=OR			
L2	L1 with (platelet or thrombosis)	3	L2
L1	integrin with (aptamer or nucleic adj2 ligand)	10	L1

END OF SEARCH HISTORY

Logging in to Dialog

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--File 515 D&B Dun's Electronic Business Directory is now online completely updated and redesigned. For details, see HELP NEWS 515.
***
--File 990 - NewsRoom now contains May 2002 to present records. File 993 - NewsRoom archive contains 2002 records from January 2002-April 2002. To search all 2002 records, BEGIN 990,993 or B NEWS2002.
***
--Alerts have been enhanced to allow a single Alert profile to be stored and run against multiple files. Duplicate removal is available across files and for up to 12 months. The Alert may be run according to the file's update frequency or according to a custom calendar-based schedule. There are no additional prices for these enhanced features. See HELP ALERT for more information.
***
--U.S. Patents Fulltext (File 654) has been redesigned with new search and display features. See HELP NEWS 654 for information.
***
--Connect Time joins DialUnits as pricing options on Dialog. See HELP CONNECT for information.
***
--CLAIMS/US Patents (Files 340,341, 942) have been enhanced with both application and grant publication level in a single record. See HELP NEWS 340 for information.
***
--SourceOne patents are now delivered to your email inbox as PDF replacing TIFF delivery. See HELP SOURCE1 for more information.
***
--Important news for public and academic libraries. See HELP LIBRARY for more information.
***
--Important Notice to Freelance Authors--
See HELP FREELANCE for more information
***
For information about the access to file 43 please see Help News43.
***
NEW FILES RELEASED
***Dialog NewsRoom - Current 3-4 months (File 990)
***Dialog NewsRoom - 2002 Archive (File 993)
***Dialog NewsRoom - 2001 Archive (File 994)
***Dialog NewsRoom - 2000 Archive (File 995)
***TRADEMARKSCAN-Finland (File 679)
***TRADEMARKSCAN-Norway (File 678)
***TRADEMARKSCAN-Sweden (File 675)

```
                    ***
UPDATING RESUMED
***Delphes European Business (File 481)
                    ***
RELOADED
***D&B Dun's Electronic Business Directory (File 515)
***U.S. Patents Fulltext 1976-current (File 654)
***Population Demographics (File 581)
***Kompass Western Europe (File 590)
***D&B - Dun's Market Identifiers  (File 516)

REMOVED
CSA Files:
***Abstracts in New Technologies and Engineering (File 238)
***Aerospace Database (File 108)
***Aluminium Industry Abstracts (File 33)
***Applied Social Sciences Index and Abstracts (File 232)
***Aquatic Sciences and Fisheries Abstracts (File 44)
***ARTbibliographies Modern (File 56)
***Ceramic Abstracts (File 335)
***Conference Papers Index (File 77)
***Engineered Materials Abstracts (File 293)
***ISMEC: Mechanical Engineering Abstracts (File 14)
***Life Sciences Collection (File 76)
***Linguistics and Language Behavior Abstracts (File 36)
***LISA (Library & Information Science Abstracts) (File 61)
***Materials Business File (File 269)
***METADEX: Metals Science (File 32)
***Oceanic Abstracts (File 28)
***Pollution Abstracts (File 41)
***Sociological Abstracts (File 37)
***Water Resources Abstracts (File 117)
Other files:
***Chicago Tribune (File 632)
***Fort Lauderdale Sun Sentinel (File 497)
***The Orlando Sentinel (File 705)
***Newport News Daily Press (File 747)
***U.S. Patents Fulltext 1980-1989 (File 653)
***Washington Post (File 146)
***Books in Print (File 470)
***Court Filings (File 793)
***Publishers, Distributors & Wholesalers of the U.S. (File 450)
***State Tax Today (File 791)
***Tax Notes Today (File 790)
***Worldwide Tax Daily (File 792)

***New document supplier***
IMED has been changed to INFOTRIE (see HELP OINFOTRI)


     >>> Enter BEGIN HOMEBASE for Dialog Announcements <<<
     >>>    of new databases, price changes, etc.      <<<
                    ****
* **                                    **

File   1:ERIC  1966-2002/Dec 13
       (c) format only 2002 The Dialog Corporation

      Set  Items  Description
      ---  -----  -----------
Cost is in DialUnits
? dialog
>>>'IALOG' not recognized as set or accession number
```

```
? b 410
       29dec02 12:30:20 User208652 Session D486.1
            $0.58    0.165 DialUnits File1
     $0.58  Estimated cost File1
     $0.21  TELNET
     $0.79  Estimated cost this search
     $0.79  Estimated total session cost   0.165 DialUnits

File 410:Chronolog(R)  1981-2002/Nov
       (c) 2002 The Dialog Corporation

      Set  Items  Description
      ---  -----  -----------
? set hi ;set hi
HILIGHT set on as ''
HILIGHT set on as ''
? b 411
       29dec02 12:30:45 User208652 Session D486.2
            $0.00    0.070 DialUnits File410
     $0.00  Estimated cost File410
     $0.09  TELNET
     $0.09  Estimated cost this search
     $0.88  Estimated total session cost   0.234 DialUnits
File 411:DIALINDEX(R)

DIALINDEX(R)
   (c) 2002 The Dialog Corporation plc

*** DIALINDEX search results display in an abbreviated ***
*** format unless you enter the SET DETAIL ON command. ***
? sf allbiosci
   You have 74 files in your file list.
   (To see banners, use SHOW FILES command)
? s integrin(9n)(aptamer or nucleic(2w)ligand)

Your SELECT statement is:
   s integrin(9n)(aptamer or nucleic(2w)ligand)

          Items   File
          -----   ----
       Examined  50 files
              1   357: Derwent Biotech Res.__1982-2002/Dec W4
              2   399: CA SEARCH(R)_1967-2002/UD=13726

   2 files have one or more items; file list includes 74 files.

? save temp integ
Temp SearchSave "TDINTEG" stored
? b n1:n2
>>>You must first use the RANK FILES command (RF)
? rf
Your last SELECT statement was:
   S INTEGRIN(9N)(APTAMER OR NUCLEIC(2W)LIGAND)

Ref        Items   File
---        -----   ----
N1             2   399: CA SEARCH(R)_1967-2002/UD=13726
N2             1   357: Derwent Biotech Res.__1982-2002/Dec W4
N3             0     2: INSPEC_1969-2002/Dec W3
N4             0     5: Biosis Previews(R)_1969-2002/Dec W4
N5             0     6: NTIS_1964-2002/Dec W5
N6             0     8: Ei Compendex(R)_1970-2002/Dec W3
N7             0    10: AGRICOLA_70-2002/Dec
```

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N8              0    29: Meteor.& Geoastro.Abs._1970-2002/Jul
N9              0    31: World Surface Coatings Abs_1976-2002/Dec
N10             0    34: SciSearch(R) Cited Ref Sci_1990-2002/Dec W5
   2 files have one or more items; file list includes 74 files.

        - Enter P or PAGE for more -
? b n1:n2
       29dec02 12:33:31 User208652 Session D486.3
            $1.49    0.852 DialUnits File411
     $1.49  Estimated cost File411
     $0.65  TELNET
     $2.14  Estimated cost this search
     $3.02  Estimated total session cost   1.086 DialUnits

SYSTEM:OS  - DIALOG OneSearch
  File 399:CA SEARCH(R)  1967-2002/UD=13726
         (c) 2002 American Chemical Society
*File 399: Use is subject to the terms of your user/customer agreement.
Alert feature enhanced for multiple files, etc. See HELP ALERT.
  File 357:Derwent Biotech Res.  _1982-2002/Dec W4
         (c) 2002 Thomson Derwent & ISI
*File 357: File is now current.  See HELP NEWS 357.
Alert feature enhanced for multiple files, etc. See HELP ALERT.

      Set  Items  Description
      ---  -----  -----------
? exs
Executing TDINTEG
Hilight option is not available in file(s) 399
HILIGHT set on as '%'
          11373  INTEGRIN
            654  APTAMER
         494545  NUCLEIC
          89247  LIGAND
            307  NUCLEIC(2W)LIGAND
      S1      3  INTEGRIN(9N)(APTAMER OR NUCLEIC(2W)LIGAND)
? t 5/all
>>>'ALL' not allowed as format type
? t 1/5/all

 1/5/1     (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 2002 American Chemical Society. All rts. reserv.

  134157589    CA: 134(12)157589g    PATENT
  Nucleic acid ligands to hepatocyte growth factor or its receptor c-met
and to integrins and their use in diagnosis and therapy
  INVENTOR(AUTHOR): Ruckman, Judy; Gold, Larry; Stephens, Andrew; Janjic,
Nebojsa; Rabin, Ross; Lochrie, Michael
  LOCATION: USA
  ASSIGNEE: Nexstar Pharmaceuticals, Inc.
  PATENT: PCT International ; WO 200109159 A1  DATE: 20010208
  APPLICATION: WO 2000US20139 (20000724) *US 364543 (19990729) *US 364539
(19990729)
  PAGES: 226 pp.  CODEN: PIXXD2  LANGUAGE: English  CLASS: C07H-021/04A;
C07H-021/02B; A61K-048/00B; C12Q-001/68B; C12N-015/85B; C12N-015/86B
  DESIGNATED COUNTRIES: AE; AG; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; BZ;
CA; CH; CN; CR; CU; CZ; DE; DK; DM; DZ; EE; ES; FI; GB; GD; GE; GH; GM; HR;
HU; ID; IL; IN; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MA;
MD; MG; MK; MN; MW; MX; MZ; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL;
TJ; TM; TR; TT; TZ; UA; UG; UZ; VN; YU; ZA; ZW; AM; AZ; BY; KG; KZ; MD; RU;
TJ; TM  DESIGNATED REGIONAL: GH; GM; KE; LS; MW; MZ; SD; SL; SZ; TZ; UG; ZW
; AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE;
```

BF; BJ; CF; CG; CI; CM; GA; GN; GW; ML; MR; NE; SN; TD; TG
SECTION:
CA201012 Pharmacology
CA203XXX Biochemical Genetics
IDENTIFIERS: aptamer scatter factor receptor integrin SELEX, diagnosis therapy scatter factor receptor integrin aptamer
DESCRIPTORS:
Integrins...
.alpha.IIb.beta.3; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
Integrins...
.alpha.v.beta.3; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
Platelet-derived growth factors...
aptamers for, pharmaceuticals contg.; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
DNA... Nucleic acids... RNA...
aptamers; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
Transforming growth factors...
.beta.-, aptamers for, pharmaceuticals contg.; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
Integrins...
.beta.3; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
Eye,disease...
diabetic retinopathy; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
Uterus,disease...
endometriosis; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
Eye,disease...
macula, degeneration; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
Angiogenesis inhibitors... Anticoagulants... Antirheumatic agents... Antitumor agents... Hepatocyte growth factor receptors... Hepatocyte growth factor... Integrins... Osteoporosis... Psoriasis...
nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
Artery,disease...
restenosis; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
Eye,disease...
retinopathy; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
Brain,disease...
stroke; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
CAS REGISTRY NUMBERS:
106096-93-9 127464-60-2 148348-15-6 aptamers for, pharmaceuticals contg.; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
324085-57-6 324085-58-7 324085-59-8 324085-60-1 324085-61-2
325175-02-8 325175-03-9 325175-04-0 325175-05-1 325175-06-2
325175-07-3 325175-08-4 325175-09-5 325175-10-8 325175-11-9
325175-12-0 325175-13-1 325175-14-2 325175-15-3 325175-16-4
325175-17-5 325175-18-6 325175-19-7 325175-20-0 325175-21-1

325175-22-2 325175-23-3 325175-24-4 325175-25-5 325175-26-6
325175-27-7 325175-28-8 hepatocyte growth factor-binding aptamer; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
227083-82-1 324085-62-3 324824-87-5 324824-88-6 324824-89-7
324824-90-0 324824-91-1 324824-92-2 324824-93-3 324824-94-4
324824-95-5 324824-96-6 324824-97-7 324824-98-8 324824-99-9
324825-00-5 324825-01-6 324825-02-7 324825-03-8 324825-04-9
324825-05-0 324825-06-1 324825-07-2 324825-08-3 324825-09-4
324825-10-7 324825-11-8 324825-12-9 324825-13-0 324825-14-1
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325175-57-3 325175-58-4 325175-59-5 325175-60-8 325175-61-9
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325175-77-7 325175-78-8 325175-79-9 325175-80-2 325175-81-3
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325496-72-8 325496-73-9 325496-74-0 325496-75-1 325496-76-2
325496-77-3 325496-78-4 325496-79-5 325496-80-8 325496-81-9
325496-82-0 325496-83-1 325496-84-2 325496-85-3 325496-86-4
325496-87-5 325496-88-6 325496-89-7 325496-90-0 325496-91-1
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325497-37-8 325497-38-9 325497-39-0 325497-40-3 325497-41-4
325497-42-5 325497-43-6 325497-44-7 325497-45-8 325497-46-9
325497-47-0 325497-48-1 325497-49-2 325497-50-5 325497-51-6
325497-52-7 325497-53-8 325497-54-9 325497-55-0 325497-56-1
325497-57-2 325497-58-3 325497-59-4 325497-60-7 325497-61-8
325497-62-9 325497-63-0 325497-64-1 325497-65-2 325497-66-3
325497-67-4 325497-68-5 325497-69-6 325497-70-9 325497-71-0
325497-72-1 325497-73-2 325497-74-3 325497-75-4 325497-76-5
325497-77-6 325497-78-7 325497-79-8 325497-80-1 325497-81-2
325497-82-3 325497-83-4 325497-84-5 325497-85-6 nucleotide sequence; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in diagnosis and therapy
172493-55-9 172493-57-1 186556-22-9 249264-67-3 249566-21-0
317403-78-4 324086-44-4 324086-45-5 324086-46-6 324086-47-7
324086-48-8 324086-49-9 324086-50-2 324086-51-3 324086-52-4
324086-53-5 324086-54-6 324086-55-7 324086-56-8 324086-57-9
unclaimed nucleotide sequence; nucleic acid ligands to hepatocyte growth factor or its receptor c-met and to integrins and their use in

diagnosis and therapy

1/5/2 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

131182912 CA: 131(14)182912c JOURNAL
Cytoplasmic RNA modulators of an inside-out signal-transduction cascade
AUTHOR(S): Blind, Michael; Kolanus, Waldemar; Famulok, Michael
LOCATION: Institut fur Biochemie, Ludwig-Maximilians-Universitat Munich, Munich, Germany, 81377
JOURNAL: Proc. Natl. Acad. Sci. U. S. A. DATE: 1999 VOLUME: 96
NUMBER: 7 PAGES: 3606-3610 CODEN: PNASA6 ISSN: 0027-8424 LANGUAGE: English PUBLISHER: National Academy of Sciences
SECTION:
CA213006 Mammalian Biochemistry
IDENTIFIERS: RNA aptamer binding CD18 cell adhesion ICAM1, integrin LFA1 binding RNA aptamer cell adhesion
DESCRIPTORS:
RNA...
aptamers; cytoplasmic expression of CD18 integrin-binding RNA aptamers reduces inducible cell adhesion to ICAM-1
Integrins...
.beta.2, CD18; cytoplasmic expression of CD18 integrin-binding RNA aptamers reduces inducible cell adhesion to ICAM-1
Cell adhesion... Molecular association... Molecular recognition...
cytoplasmic expression of CD18 integrin-binding RNA aptamers reduces inducible cell adhesion to ICAM-1
Signal transduction,biological...
cytoplasmic RNA modulators of inside-out signal-transduction cascade
Cell adhesion molecules...
ICAM-1 (intercellular adhesion mol. 1); cytoplasmic expression of CD18 integrin-binding RNA aptamers reduces inducible cell adhesion to ICAM-1
CAS REGISTRY NUMBERS:
239121-73-4P 239121-74-5P 239121-75-6P cytoplasmic expression of CD18 integrin-binding RNA aptamers reduces inducible cell adhesion to ICAM-1

1/5/3 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0283756 DBR Accession No.: 2002-05603 PATENT
Novel non-naturally occurring %nucleic% acid (RNA) %ligand% to a beta-3 type %integrin%, useful in the treatment of cancer and thrombosis - RNA ligand preparation and purification by Systematic Evolution of Ligands by EXponential Enrichment and antibody, DNA library, DNA primer and reverse transcription-polymerase chain reaction for genetherapy
AUTHOR: RUCKMAN J; GOLD L; STEPHENS A; JANJIC N
PATENT ASSIGNEE: GILEAD SCI INC 2001
PATENT NUMBER: US 6331394 PATENT DATE: 20011218 WPI ACCESSION NO.: 2002-121160 (200216)
PRIORITY APPLIC. NO.: US 606477 APPLIC. DATE: 20000629
NATIONAL APPLIC. NO.: US 364543 APPLIC. DATE: 19990729
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A new purified and isolated non-naturally occurring %nucleic% acid %ligand% (I) to an %integrin%. DETAILED DESCRIPTION - (I) is preferably identified by a method (M1) comprising: (1) preparing a candidate mixture of nucleic acids; (2) contacting the candidate mixture of nucleic acids with the integrin, where the nucleic acids having an increased affinity to the integrin, relative to the candidate mixture, may be separated from the remainder

of the candidate mixture; (3) separating the increased affinity nucleic acids from the remainder of the candidate mixture; and (4) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acids with relatively higher affinity and specificity for binding to the %integrin%, so that a %nucleic% acid %ligand% of the %integrin% may be identified. An INDEPENDENT CLAIM is also included for the isolation of (I) comprising (M1) with isolation of (I) after its identification. BIOTECHNOLOGY - Preparation: (I) was prepared by (M1) and then isolated (claimed). Preferred Method: (M1) is known as the SELEX method (Systematic Evolution of Ligands by EXponential Enrichment) and is described in U.S. pat. No. 5,475,096 Nucleic Acid Ligands and U.S. Pat. No. 5,270,163 (see also WO 91/19813) entitled Methods for identifying Nucleic Acid Ligands. Preferred Integrin: The integrin is a beta3 and is selected from the group comprising alphaIIbbeta3 integrin and alphavbeta3 integrin, preferably alphaIIb.beta3 .The integrin is attached through either covalent or non-covalent bonds to a solid support, e.g., a bead, and steps (b)-(c) of (M1) take place on the surface of the solid support. Preferred Nucleic Acid Ligand: (I) is a purified and non-naturally occurring RNA ligand to an integrin and is selected from a group of 113 fully defined nucleotide sequences given in the specification. (I) is single stranded and comprises 2'-fluoro (2'-F) modified nucleotides. Preferred Methods: The candidate mixture of nucleic acids is comprised of single stranded nucleic acids, i.e., ribonucleic and deoxyribonucleic acids, where the ribonucleic acids comprise 2'-F (2'-fluoro) modified ribonucleic acids. ACTIVITY - Cytostatic; thrombolytic; cardiant; antidiabetic; ophthalmological; antipsoriatic; gynecological. No supporting data given. MECHANISM OF ACTION - Nucleic acid ligand for integrins; alphaIIbbeta3 and alphavbeta3 binding inhibitor. To test whether aptamer 17.16 (a fully defined 84 RNA sequence given in the specification) could block the ligand binding site of alphaIIbbeta3 and alphavbeta3, purified vitronectin and fibrinogen were biotinylated and incubated with one or both of the immobilized integrins in the presence or absence of varying concentrations of the aptamer or a non-binding control RNA. This was done as follows: purified integrin ligands, vitronectin and fibrinogen, were biotinylated according to (Smith et al. (1990) J. Biol. Chem. 265:12267-71). Biotinylated proteins were dialyzed into phosphate-buffered saline and 96-well microtiter plates were coated with either alphaIIbbeta3 or alphavbeta3 and blocked with BSA. A fixed concentration of biotinylated ligand (fibrinogen: 6 nM final; vitronectin: 10 nM final) was pre-mixed in binding buffer with varying concentrations of aptamer, control RNA, cyclic RGD peptide, antibody, or unmodified ligand. The mixtures were incubated in the integrin-coated wells for 60 minutes at room temperature. After washing, bound biotinylated ligand was detected by addition of 100 microl/well 1:500 dilution streptavidin-alkaline phosphatase conjugate (Calbiochem) (30 minutes at room temperature) followed by 100 microl/well p-nitrophenyl phosphate. Absorbance was read at 405 um. The data were fit to an equation that describes mutually exclusive binding of two ligands to a single target species (Gill et al. (1991) J. Mol. Biol. 220:307-24). The concentration of competitor that inhibited 50% of the maximum signal above background (IC50) was determined from the fitted curve. Known ligand binding inhibitors, including an RGD peptide and the alphavbeta3-specific antibody LM609, were included as positive controls for the assay. Aptamer 17.16 inhibited the binding interaction with an IC50 of 4.7 nM while the control RNA showed no inhibition. By comparison, the IC50 of RGD peptide inhibition was 1.4 nM and that of LM609 was 2.7 nM. Unmodified vitronectin inhibited the binding of the biotinylated material with an IC50 of 59 nM. Similar data were obtained for aptamer inhibition of fibrinogen binding to alphavbeta3 and for fibrinogen binding to alphaIIbbeta3. IC50 values for alphavbeta3 inhibition were 17.16, 9.5 nM; control RNA, not measurable; RGD peptide, 1.0 nM; LM609, 6.3 nM; unmodified fibrinogen, 43 nM. IC50

values for alphaIIbbeta3 inhibition were: 17.16, 6.5 nM; control RNA, not measurable; RGD peptide, 21 nM; unmodified fibrinogen, 15 nM. %Aptamer% 17.16 is an effective competitor of beta3 %integrin% ligand binding and, on a molar basis, has an inhibitory potency nearly equivalent to that of a bivalent antibody. USE - (I) is especially useful as an inhibitor of alphaIIbbeta3 and alphavbeta3 integrins and can be used to inhibit tumor growth and metastasis. They can also be used to treat ocular diseases including diabetic retinopathy, retinopathy of prematurity, and macular degeneration. Other diseases treated include endometriosis and psoriasis In addition, (I) may useful in the treatment of thrombosis and cancer, and can be used as a diagnostic agent for thrombosis. (I) can also be used to treat acute coronary syndromes such as unstable angina or myocardial infarction. Radiolabeled (I) to platelet-expressed integrins can be administered to individuals who are to undergo major surgery, or have suffered major trauma. (I) can function as imaging agents for the detection of thrombi, by showing sites in the body where large aggregations of platelets are present. If a thrombosis is detected by radioimaging at a critical site in the body, then anticoagulant and thrombolytic treatment, including treatment with (I), can be given locally. The advantage of using (I) as an imaging agent is that the anticoagulant and thrombolytic treatments, which can cause harm if administered prophylactically by allowing internal bleeding to continue without efficient clotting, can be given only to those individuals who definitely have a dangerous thrombosis. Moreover, these treatments can be specifically injected at the site where the thrombosis has been detected by the nucleic acid ligand, instead of injecting higher concentrations into the bloodstream in the hope that some active agent will be carried to all potential sites of thrombosis. ADMINISTRATION - No details given. ADVANTAGE - (I), because of its specificity for the active, ligand-binding conformation of the integrin alphaIIbbeta3, may reduce the risk of bleeding complications associated with the existing anti-clotting therapies. Given the role of integrins in various disease states, the availability of high specificity inhibitors of integrins such as (I) is a particular advantage. EXAMPLE - (I) was generated using the SELEX (Systematic Evolution of Ligands by EXponential Enrichment). A DNA template library of sequence (5'-ttatacgactcactatagggagacaagaataaacgctcaannnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn nnnnnnnttcgacaggaggctcacaacaggc-3') , was prepared by chemical synthesis, containing a T7 RNA polymerase promoter and 40 n residues (n = a,g,t, or c). A short DNA primer (5'-gcctgttgtgagcctcctgtcgaa-3') (3N8) was annealed to the template and extended using Klenow DNA polymerase. The double-stranded DNA product served as a product for T7 RNA polymerase transcription to generate a library of random-sequence RNAs. 2'-fluoro-CTP and -UTP were used in place of the 2'-OH-pyrimidines. For application of the SELEX process to alphavbeta3 integrin, the purified protein was diluted 1000-fold from detergent-containing storage buffer into 50 mM MES (2-(N-morpholino)ethanesulfonic acid), pH 6.1, 150 mM NaCl, 2 mM CaCl2 to a final concentration of approximately 0.2 microg/ml. 3.2 micropolystyrene particles were added to the diluted protein and the mixture was rotated overnight at 4degrees C. The beads were collected by centrifugation and blocked by incubation in 3% BSA in MES buffer for one hour at room temperature. Blocked beads were washed by resuspension in binding buffer (50 mM Tris. HCl, pH 7.4 (at 37degrees C), 145 mM NaCl, 4 mM KCl, 1 mM MgCl2 2 mM CaCl2, 0.1 mM MnCI2, 0.01% BSA). For one round of selection, integrin-coated beads were mixed with RNA and rotated at 37degrees C for 4 hours to allow equilibration of the RNA with the immobilized protein. The beads were then collected by centrifugation and washed 5 times in binding buffer. RNAs that remained bound to the beads were eluted overnight at 37degreesC in binding buffer plus 100 microM cyclic RGD peptide (cRGD). Eluted RNAs were extracted with phenol, then chloroform:isoamyl alcohol (24:1), and

ethanol precipitated. The RNA pellet was resuspended and annealed to primer 3N8 for reverse transcription. The cDNA pool was amplified by the polymerase chain reaction using the 3N8 primer and primer 5N8 (5'-taatacgactcactatagggagacaagaataaacgctcaa-3'). Transcription of the PCR product generated an RNA pool to initiate a new round of selection. For the first round of selection 1 nmol of RNA (approximately 6 x 1014 sequences) was incubated at 2 microM concentration with a volume of bead suspension equivalent to 50 pmol of protein (assuming all the integrin had adsorbed to the beads). In subsequent rounds, the concentration of RNA and protein-coated beads were both reduced to demand higher affinity binding interactions. The affinity of individual RNAs and RNA pools for alphavbeta3 was determined by titration of biotinylated RNA with a small quantity of immobilized integrin. Bound RNA was detected through the biotin moiety. Biotinylated RNA was prepared according to standard transcription protocols, but including a 5-fold molar excess of a 5'-biotin-modified GMP over GTP in the reaction mixture. Methods for synthesizing 5'-biotin-modified guanosine nucleotides are described in WO 98/30720 entitled Bioconjugation of Oligonucleotides. The modified nucleotide was incorporated at the 5' end of the transcript in proportion to its representation in the guanosine pool. 96-well microtiter plates were coated overnight at 4degreesC with 100 microl purified alphavbeta3 at a concentration of 0.25 microg/ml in 20 mM Tris.HCl, pH 7.5, 150 mM NaCl, 1 mM MgCl2 2 mM CaCl2 , 0.1 mM MnCl2. Coating concentrations were 0.8 microg/ml for alphaIIbbeta3 and 0.3 microg/ml for alphavbeta3. Wells were blocked with 200 microl of a solution of 3% BSA in the same buffer (1 hour at room temperature) then rinsed 3 times with 200 microl binding buffer. Individual RNAs or RNA pools were denatured briefly at 93degrees C in binding buffer without divalent cations or BSA, then serially diluted in the same buffer. 50 microl binding buffer containing 2x-concentrations of divalent cations and BSA were added to each well, followed by 50 microl RNA dilution. RNAs were allowed to incubate in the integrin-coated wells at 37degrees C for 30-60 minutes. Unbound RNAs were removed by 3 rapid washes in binding buffer. To detect bound RNA, 100 microl of a 1:2500 dilution in binding buffer of streptavidin-alkaline phosphatase conjugate were incubated in each well for 30 minutes at room temperature, followed by three rapid washes, as above. 100 microl/well p-nitrophenyl phosphate was added and incubated at room temperature for 30 minutes. Color development was monitored by absorbance at 405 nm. Binding data were fit to an equation that describes the fraction of RNA or protein bound as a function of KD, and the total concentrations of RNA and protein in the binding reaction for both monophasic and biphasic binding behavior (Green et al. (1996) Biochem. 35:14413-24). A control RNA corresponding to a sequence-scrambled version (I) (5'-gggagacaagaauaaucgcucaacguugaaugcugc auuauggaguauugaccgcuacaucccuucgaca ggaggcucacaacaggc-3') was used to monitor non-specific binding of RNA under the conditions of the assay. After seven rounds of the SELEX process, the amount of RNA specifically bound to the integrin-coated beads had increased substantially (data not shown). Although immobilized alphavbeta3 showed no detectable affinity for random sequence RNA, the Round 7 RNA pool bound with an equilibrium dissociation constant (KD) of approximately 4x 10-7 M. The Round 7 affinity-enriched pool was cloned and sequences were determined for individual molecules in the mixture. Of 92 sequences obtained, 35 (38%) were very highly related to one another, in many cases differing at no more than a single base position. These sequences are collectively referred to as Family 1. It is likely that many if not most of these RNAs derived from a single precursor as a result of errors in replication during the RT and PCR steps. Another 25 sequences (27%) shared a short motif (CCUGCC) that defined a second sequence family (Family 2). The remaining 32 sequences (35%) were not obviously related to sequences in Families 1 or 2 and were thus termed orphan sequences. The large percentage of orphan sequences in the round 7 pool

suggested that a great deal of sequence complexity remained in the population. Therefore, the SELEX process was continued in the hope of further enriching for high affinity sequences whose representation in the round 7 pool may have been low. Indeed, a substantial improvement in the affinity of the RNA pool was observed after 8 additional rounds of affinity selection (Round 15). No further improvement was seen after two more rounds of selection (Round 17), so clones were isolated from the Round 15 and Round 17 RNA pools and the sequences of individual isolates were compared to those obtained at Round 7. Twenty-seven of 39 sequences derived from the Round 15 pool (69%) were members of the highly conserved sequence family, Family 1. Three sequences (8%) could be grouped with Family 2 and 9 sequences (23%) were orphans. All of the 18 sequences isolated from the Round 17 pool were members of sequence Family 1. Thus, in this case, additional rounds of the SELEX process served to focus the RNA population on a single high affinity sequence family that was already predominate at Round 7. (50 pages)

DESCRIPTORS: beta-3-type integrin-specific RNA ligand prep., purification, SELEX method, antibody, DNA library, DNA primer, reverse transcription-polymerase chain reaction, appl. cytostatic, thrombolytic, cardiant, antidiabetic,ophthalmological, antipsoriatic, gynecological act., gene therapy protein hybridization DNA amplification DNA sequence RNA sequence (21, 23)

SECTION: THERAPEUTICS-Gene Therapy-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; DISEASE-Cardiovascular-DISEASE-Other Diseases; DISEASE-Endocrine/Metabolic System-DISEASE-Cancer

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